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Original Article

Influence of passive ultrasonic tip activation at different levels on the depth of sealer penetration: An *in vitro* study

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Abstract

Introduction: The aim of this study was to compare the effect between different levels of ultrasonic tip activation on the depth of epoxy resin-based sealer (AH plus) dentinal tubules penetration.

Materials and Methods: Extracted single-rooted premolars ($n = 60$) were randomly allocated into three groups and instrumented following the same protocol. Group 1 (control), the sealer was mixed with 0.1% Rhodamine B dye and placed using size 20 K-file. In Group 2, the sealer was passively activated using ultrasonic tip (ISO 25) 10 s mesiodistally and buccolingually at 2 mm from the apex. In Group 3, the sealer was activated in a similar manner at 4 mm from the apex. Samples were sectioned horizontally at 2 mm, 4 mm, and 6 mm from the apex and analyzed using a stereomicroscope for tubular dentine sealer penetration. The cross-sectional area (μm^2) was measured with software to get the percentage of sealer penetration, and presences of voids were recorded.

Results: Significant lesser percentage of sealer penetration into the dentinal tubules was observed between the control group and both the experimental groups ($P = 0.00$) at 2 mm, 4 mm, and 6 mm from the apex. There was no significant difference in the percentage of sealer penetration into the dentinal tubules between both the experimental groups ($P > 0.05$). The presence of voids between all groups was not statistically significant ($P > 0.05$).

Conclusion: Passive ultrasonic activation of sealer placement resulted in deeper sealer penetration into the dentinal tubules even at a higher level of tip activation (4 mm).

Keywords: Depth of penetration, epoxy resin sealers, obturation, sonic, ultrasonic

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INTRODUCTION

Three-dimensional obturation is important to provide an impermeable, fluid-tight seal to prevent oral, and apical microleakage.[1] One important component of the obturation is the placement of sealer. Conventional hand placement techniques of root canal sealers involve the use of reamers, absorbent paper points, lentulo spiral, and gutta-percha cones.[2] The limitations of all the techniques are the inability of the sealer to penetrate into canal

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irregularities and anatomical variations. Sealers may not be able to penetrate deep into dentinal tubules especially at the apical third of the root canal where most of the canal irregularities, i.e., accessory canal, apical delta, and anastomosis exist.[3]

The presence of voids and porosities in an unsatisfactory root canal filling and could lead to lower success rates compared to satisfactory root fillings.[4] Bacteria are the main etiological factor for root canal failure, and it has been shown that they might be still present even after thorough chemomechanical preparation.[5,6] Previous research has discovered that bacteria can penetrate up to 250 μm into the dentinal tubules.[7] The increased penetration of root canal sealer into dentinal tubules will further enhance the antimicrobial activity, thus decreasing the bacterial load within the root canal system.[8,9] Furthermore, the mechanical interlocking of the sealer plug inside the tubules can further improve retention as well as the sealing ability of the sealer.[10]

Numerous techniques have been used to improve the distribution of root canal sealers within the root canal system. *In vitro* studies have shown that ultrasonically activated sealer placement showed a significantly higher percentage of depth of sealer penetration compared to other methods of sealer placement techniques.[11-13] Due to the limitations of conventional sealer placement techniques, there is a need to improve the efficacy of sealer penetration into dentinal tubules, especially in the apical third of the root canal system.[14] The aim of this study was to compare the depth of epoxy resin-based sealer (AH plus) penetration into the dentinal tubules with and without ultrasonic activation of the sealer. The effect of different levels of ultrasonic tip activation on the depth of sealer penetration into the dentinal tubules was investigated.

MATERIALS AND METHODS

Sixty freshly extracted human mandibular second premolar teeth extracted for orthodontic purposes were randomly divided into three groups. The sample size was calculated using sample size calculator (CTSI/UCSF-2013) and the sample size for each group was standardized to 20. Ethical approval was obtained from the Universiti Kebangsaan Malaysia (UKM) Ethics Committee (UKM 1.5.3.5/244/DD/2015/002 (2)). Intact single-rooted mandibular second premolar teeth with complete root formation, sound, noncarious with no sign of resorption or previous root canal treatment were selected. Preoperative radiograph was taken and teeth with structural defects, caries, restorations, calcified canal, having open apices or apical foramen larger than 0.4 mm, bifurcated canal, curvature more than 10°, having S-shaped or C-shaped canal were excluded from this study. Teeth were stored in 10% formalin and kept within 1 month to avoid prolonged storage. Teeth were decoronated using diamond bur TC-11 (Mani, Japan) and the length of all teeth was standardized at 15 mm. The working lengths were established by subtracting 0.5 mm from the point where a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) penetrated the apical foramen. The teeth were then mounted in a silicone index and held by a jig during chemomechanical preparation. Canal cleaning and shaping were performed using Protaper Universal rotary files (Dentsply-Maillefer, Ballaigues, Switzerland). The canals were prepared using the S1 and S2 files, followed by the finishing files from size F1, size F2, size F3, and finally size F4. After the use of each instrument, the canals were irrigated with 2.5% sodium hypochlorite. Passive sonic irrigation was performed using EndoActivator with medium size tip (25/0.04) (Dentsply Tulsa Dental Specialties, California, USA) and a final rinse of 2 ml 17% ethylenediaminetetraacetic acid (Meta Biomed, Co. Ltd., Chungbuk, Korea) was applied for 2 min. Then, the canals were flushed with 2.5% of sodium hypochlorite followed by saline solution. Paper points size 40 (Dentsply Maillefer, Ballaigues, Switzerland) were used to dry the canals.

AH Plus sealer (De Trey, Dentsply, Konstanz, Germany) was mixed with Rhodamine B dye (Sigma-Aldrich, St. Louis, MO, USA) which is magenta-pink in color to achieve 0.1% concentration.[15] A size 20 k-file was used to place the sealer into all the canals, using a counterclockwise rotation.[16] For the control group (Group 1), all canals were obturated right after sealer placement. The ultrasonic tip was placed at different levels for Group 2 and Group 3. For Group 2, the sealer was activated using a size 25 K-file ultrasonic tip attached to the 120° Endochuck (EMS, Switzerland) using an EMS MiniMaster motor (Piezon®, E. M. S. Electro Medical Systems S.A., Switzerland). A rubber stopper was placed, and the ultrasonic tip was inserted at 2 mm short from the working length. The ultrasonic file was activated using "endo" mode without water at 30% low power setting. The file was activated for 10 s in a buccolingual direction and 10 s in a mesiodistal direction of the root canal. The same method of sealer placement was carried out in Group 3, except that the file was activated 4 mm short from the working length. After placement of sealers, all 60 canals were obturated using cold lateral condensation technique using prefitted size F4 ProTaper Universal (Dentsply-Maillefer, Ballaigues, Switzerland) gutta-percha points and accessory gutta-percha points. Gutta-percha was cut 3 mm from the orifice and condensed vertically with a heated plugger, and Cavit G (3M ESPE, Germany) was placed as temporary filling. The teeth were stored in an incubator at 37°C and 100% humidity for 7 days, to allow the sealer to set. During sealer placement, freshly mixed sealer was used to obturate five canals per mixture. Sealer placement method was changed with every five teeth in each group and then shifted to another group to minimize operator becoming more proficient with one particular method and to avoid fatigue as a confounding variable.[17]

Each tooth was embedded in self-cured clear acrylic (Probase®–Brazil, Ivoclar Vivadent, Liechtenstein, Germany) and sectioned horizontally in a mesiodistal direction at 2 m, 4 mm, and 6 mm from the apex using Isomet 4000 (Buhler®, Illinois, USA), which produces high polished slides. A high-resolution stereomicroscope (Zeiss Stemi 2000-C Stereo Microscope-7.7X to 1X Zoom Range, Jena, Germany) was used to observe each slice using 1.6X magnification and images of the slices were captured using AxioCam ERc5c-Zen Lite 2012 (Blue Edition) software. A single calibrated examiner measured the penetration of the pink-stain of Rhodamine B dye into the dentinal tubules of all the 180 slices. The correlation coefficient for intraexaminer reliability was 0.998.

Surface areas of penetration were demarcated using the AxioCam ERc5c-Zen Lite 2012 software. Values of dentinal tubules penetration were recorded as "a," the surface areas of the inner perimeter of the canal walls were measured by demarcating the outline of the canals on the images as label "b," and the whole cross-sectional surface areas of the root (outer perimeter) were recorded in the same manner and labeled as "c" [Figure 1]. Then, the percentages of cross-sectional area (μm^2) of sealant penetration into dentinal tubules were calculated by similar method described by Aguirre *et al.*[17] All images were also analyzed for the presence of voids at the interface between gutta-percha/sealer and the canal walls. Images were scored "0" if no voids were present and scored "1" if voids were present.

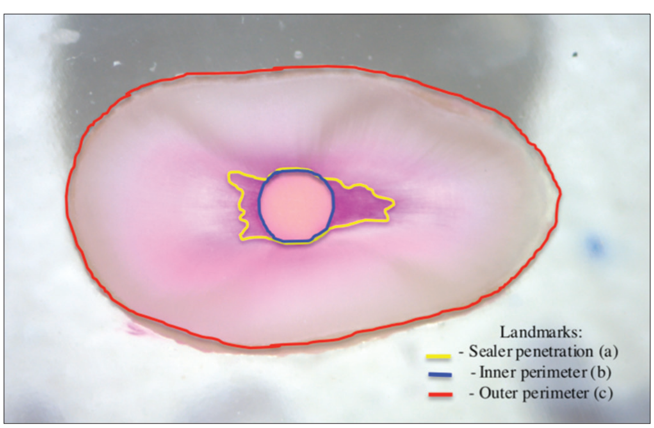

Figure 1: Image of the cross-sectional root surface showing the demarcation of the sealer penetration, inner perimeter of the canal wall and outer perimeter of the root surface

Statistical analysis was performed using Kruskal–Wallis test. *Post hoc* Mann–Whitney U-test was carried out to compare the differences between the control and Group 2 (ultrasonic activation at 2 mm), Group 2 and Group 3 (ultrasonic activation at 4 mm), and the control group and Group 3 at all levels tested. Fisher's exact test was carried out to compare the presence of voids between the control group, Group 2 and Group 3 at 2 mm, 4 mm, and 6 mm from the apex.

RESULTS

Median and interquartile range for the percentages of depth of dentinal tubules sealer penetration for Group 1, Group 2, and Group 3 at 2 mm, 4 mm, and 6 mm were calculated. There were statistically significant differences of dentinal tubule penetration of sealers at all levels between the control group, Group 2, and Group 3 (H [corrected for ties] =15.829 [2 mm], 25.997 [4 mm], and 27.74 [6 mm], df = 2, n = 60, with P = 0.000, and Cohen's effect size [f] = 0.942). Significant lower percentages of sealer penetration into the dentinal tubules were observed between the control group and both the experimental groups (P = 0.00) at 2 mm, 4 mm, and 6 mm from the apex [Table 1]. There was no statistically significant difference in the percentage of sealer penetration into the dentinal tubules between both the experimental groups, which were Group 2 and Group 3 ($P > 0.05$). The presence of voids recorded between all the groups was not statistically significant at all levels (P = 0.863).

DISCUSSION

Ultrasonic method of sealer placement in this study resulted in significantly deeper penetration of sealer into the dentinal tubules compared to manual hand sealer placement which is similar to other studies.[15] However, the difference is the manipulation of ultrasonic tip at several different levels which are at 2 mm and 4 mm that is not performed in the previous study. The results showed that the percentage of cross-sectional area (μm^2) for sealant penetration into dentinal tubules using ultrasonic was two to four times greater compared to the manual hand sealer placement when comparing the median value of control group (1.12) to the median value of Group 2 (2.73) and median value of Group 3 (4.16) at the level of 4 mm from the apex. Ultrasonic activation resulted in increased

Table 1: Result for the comparison of depths of sealer penetration using Mann–Whitney test

	Percentage of sealer penetration at 2 mm (*P*)	Percentage of sealer penetration at 4 mm (*P*)	Percentage of sealer penetration at 6 mm (*P*)
Group 1 versus Group 2	0.000*	0.000*	0.000*
Group 1 versus Group 3	0.000*	0.000*	0.000*
Group 2 versus Group 3	0.678	0.289	0.142

*There were statistically significant differences of dentinal tubules penetration of sealers at all levels between the control group, Group 2, and Group 3

penetration of sealer in this study may be attributed to the acoustic streaming field generated around an oscillating ultrasonic file as reported in previous studies.[18,19] A study showed that higher velocity of streaming patterns was recorded using smaller size files (ISO 15, 20, and 25) compared to larger size files (ISO 30 and 35).[20] A size 25 stainless steel K-file attached to Endochuck 120° (EMS, Switzerland) was used in this study to activate the sealer as it exhibited less displacement amplitudes compared to size 15 or 20 K-file. This resulted in a decreased risk of binding with the canal wall, preventing damage to the root canal and may reduce the incidence of file separation.[21] Currently, piezoelectrically powered devices have become the preferred choice compared to magnetostrictive devices as the former generates less heat.[22] Furthermore, the use of piezoelectrically powered devices in this study was limited to <15 s, which was found in other studies to not generate sufficient heat that may cause injury to the periradicular tissue.[23] Rhodamine B dye has been chosen as tracer dye to evaluate the sealer penetration in this study. Rhodamine B dye is a hydrophilic tracer dye used to determine the rate and flow direction of the sealer. Its molecular formula is $C_{28}H_{31}CIN_{2}0_{3}$ and its molecular weight is 536.1 g/mol and particle size is 610 nm.[24] It is in the form of green crystals powder, which is very soluble in water. Rhodamine B dyes fluoresce and take up a brilliant pink color on contact with water, thus could be detected easily or could be detected using fluorometers.

A recent study showed the use of the ultrasonic activation of an epoxy-amine resin-based sealer promoted greater sealer penetration even into the lateral canals.[25] However, this is the first study, which has assessed the effect of different levels of tip activation on sealer penetration by adjusting the ultrasonic tip at two different levels (2 mm and 4 mm). This study showed that ultrasonic tip activation at 4 mm away from the apex resulted in similar depth of sealer penetration into the dentinal tubules compared to ultrasonic tip activation at 2 mm from the apex. As such, ultrasonic tip activation at 4 mm away from the apex may be an acceptable approach to achieve equally satisfactory penetration of sealer into the dentinal tubules. In natural teeth, the canal tends to curve either toward the labial or palatal aspect at about the apical third level.[26] Therefore, in cases of severely curved canals, ultrasonic tip placed further away from the apex would also be better to minimize procedural errors, which would commonly occur beyond the canal curvature. The risk of iatrogenic injury in this study was also reduced with the use of file size 25, instead of smaller size files, which were reported to exhibit high incidence of file separation.[27] The incidence of ultrasonic file separation ranges between 3.3% and 10%.[27,28] The file separations were located 2–3 mm away from the ultrasonic tip, and the probability of separating a file in apical third was 33%, which is six times more likely when compared to coronal and middle thirds of the canal.[28] Ultrasonic tip activation at this length (4 mm) may also reduce the incidence of iatrogenic damage, such as instrument separation, especially in curved canals.

There was no significant difference found in the presence of voids between ultrasonic methods of sealer placement compared to manual hand placement method of sealer placement. The limitations of this study include the use of stereomicroscope, which only allows two-dimensional image evaluation at a specific level of the root canal and artifact formation in some of the samples due to structural loss during cutting of the specimen.[29] Nevertheless, the study does provide useful findings, as this is assessed the effect of different levels of tip activation on sealer penetration. For future studies, three-dimensional analysis using micro-computed tomography should be carried out to gain more in-depth view of sealer penetration.

In addition to that, patients' age and ethnicity were not taken into consideration in the sample selection, which may possibly lead to conflicting results on the presence of voids in comparison with other studies. It was found that increasing age might result in lesser penetration due to the increase in incidence of sclerosis.[30] The rate of sclerosis also may differ between different ethnicity.[31] As age and ethnicity may be associated with incidence and rate sclerosis, the results of this study may produce differing findings in terms of penetration of sealer. Therefore, for future studies, we would like to recommend inclusion of age and ethnicity of samples, as well as other factors that may impact on the condition of the dentinal tubules, and subsequent penetration of sealer.

CONCLUSION

Passive ultrasonic activation of sealer placement resulted in a significantly higher percentage of sealer penetration depth into the dentinal tubules compared to manual hand sealer placement. Placement of the ultrasonic tip 4 mm from the apex during sealer placement is safer to prevent iatrogenic damage, without compromising the quality of sealer penetration.

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Conflicts of interest

There are no conflicts of interest.

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